In response to the Examiner's objections, applicants have canceled claims 1-13, and added new claims 14-27. The new claims address each of the Examiner's objections to original claims 1-13.

The Examiner considered it unclear as to the meanings and properties of an "osteoinductive" vs. "osteogenetic" substance. The Examiner will note that the new claims contain the limitation that the protein or DNA encoding therefor is "cartilage-inducing and/or bone-inducing". Support for this change can be found on page 9, third full paragraph. As known by those of ordinary skill in the art, the term "induction" concerns the growth, differentiation and stimulation of cells. There is overlap between the meanings of the terms "osteoinductive" and "osteogenetic". However, those of skill in the art understand that proteins of the TGF-β superfamily are responsible for several different types of effects on bone cells. For example, proteins such as MP52 are known to stimulate mesenchymal aggregation and chondrogenic differentiation, just to name two of the known activities. Therefore, the term "cartilage-inducing and/or bone-inducing activity" is quite appropriate to use in this case.

The Examiner also objected to the term "fragments thereof" in claim 2. The Examiner will note that new claim 15 (which is based on original claim 2) includes the limitations that the protein fragment is a cartilage-inducing and/or bone-inducing protein fragment. Thus, only those fragments providing for cartilage and/or bone induction are covered by claim 15.

The Examiner objected to the terms "mature" and "essentially the same activity" in original claim 3.

As is clearly stated on page 12 of the specification, SEQ ID NO: 1 is the amino acid sequence for protein MP52, which is described in WO 93/16099, WO 95/04819, WO 96/01316 and Hötten et al. (1996). The beginning of the mature protein part of MP52 is described in WO 95/04819 and WO 96/01316. SEQ ID NO: 1 does not only show the mature part, but the pre-pro protein comprising the mature part, pre-pro sequences and signal peptide. One of ordinary skill in the art would have absolutely no difficulty in finding the beginning of the mature part of the protein, since it has already been known before the present application was filed.

With regard to the term "essentially the same activity", applicants have now restricted this term to "essentially the same cartilage-inducing and/or bone-inducing activity". Such activity can be clearly proven by experiments by those of ordinary skill in the art. The paragraph bridging pages 9 and 10 of the specification provide several methods for testing this activity (e.g., *in vivo* testing by induction of cartilage and/or bone after implantation of the protein with a suitable carrier matrix into rat musculature; or *in vitro* by induction of alkaline phosphase activity in ROB-C26 cells; and/or stimulation of the expression of proteins of the extracellular matrix; and/or in experiments as described by Chen et al. (1991) and/or Vukicevic et al. (1989)). Thus, there is plenty of guidance provided for one of ordinary skill in the art to determine the metes and bounds of the term "essentially the same cartilage-inducing and/or bone-inducing activity".

The Examiner also inquired as to what other monomers and dimers from what other TGF-β proteins, when fused with SEQ ID NO: 1, provide a therapeutic effect upon implantation.

Applicants can provide ample publications on heterodimers and fusion proteins with other proteins of the TGF-β superfamily, where such proteins (for example, comprising bone morphogenetic proteins) are described. Attached, applicants provide a list of abstracts which clearly show the use and pharmaceutical activity of heterodimers in the TGF-β superfamily.

Much is know about the TGF-β superfamily of proteins. These proteins exhibit, as monomers, 6 and 7 cysteine residues, respectively, which form dimers via one of the cysteine residues and intramolecular bridges via the other cysteines in order to form the "correct" three-dimensional conformation. Due to the structural similarities between them, the monomers very much conform with one another. It had long been assumed that such heterodimers could, in fact, be applied as pharmaceuticals. The attached abstracts prove that this assumption was correct.

Applicants respectfully submit that new claims 14-27 fully comply with 35 USC §112, second paragraph. Applicants respectfully request that this rejection be withdrawn.

Claims 1, 2, 6-9 and 11-13 are rejected under 35 USC §102(b) as being anticipated by Urist et al. (U.S. Patent No. 4,596,574). Urist is relied upon as teaching the use of a biodegradable porous β-tricalcium phosphate ceramic matrix

with bone morphogenetic proteins (BMP) for the slow release of protein for the purpose of inducing new bone growth.

This rejection is respectfully traversed as to new claims 14-27. Applicants have the following comments.

Urist is directed to a delivery system for BMP comprising a composition of a physiologically acceptable, biodegradable porous ceramic containing substantially pure BMP which allows the BMP to be delivered on a sustained basis to bone tissue. The porous ceramic is preferably sintered calcium phosphate, preferably tricalcium phosphate, and especially preferably β-tricalcium phosphate. The pores of the ceramic are suitable for containing effective amounts of lyophilized BMP. The BMP-porous ceramic delivery system is prepared by introducing a physiologically acceptable biodegradable porous ceramic to an aqueous BMP solution and causing the BMP to become entrapped in the ceramic's pores by evaporating the solvent or freeze-drying it.

The present claims contain the limitation that the matrix material is "bioactive". The matrix according to Urist is not bioactive. This is clearly indicated by the Example on column 4, line 57 through column 5, line 51. In this example, BMP is implanted on β-tricalcium phosphate and, as a control, β-tricalcium phosphate having no BMP thereon is implanted in muscle pockets of mice. The implants are tested after several days. In the control test, which only contains the β-tricalcium phosphate matrix, no bone growth was detected (i.e., there is no

"bioactivity"). Instead, the negative side effects of the implant, such as infectious reactions, infiltration of macrophages and multinuclear giant cells and encapsulation in fibrous connective tissue) are described. All of these side effects can be avoided by the matrix of the present invention. Applicants refer the Examiner to page 13, end of the first paragraph, of the present specification. In order to avoid the side effects experienced using the Urist matrix, it is important to achieve a corresponding crystallographic phase purity (see page 7, first paragraph, of the present specification). Consequently, the matrices of the present application are decisively different from those of Urist. Thus, the present invention could not be anticipated by or obvious over Urist.

Claims 1, 2, 4, 5, 7, 8, 10 and 11-13 are rejected under 35 USC §102(b) as being anticipated by Oppermann et al. (WO 91/05802). Oppermann is relied upon as teaching a biodegradable, biocompatible matrix and a method of making such comprising the fabrication of a recombinant protein or DNA into the pores of the matrix, wherein the pore size is from 1-100 μm.

This rejection is respectfully traversed as to new claims 14-27. Applicants have the following comments.

Oppermann et al. is directed to an osteogenic device comprising matrices containing dispersed osteogenic protein produced from recombinant DNA and capable of bone induction in allogenic and xenogenic implants. The matrix in

Oppermann comprises biocompatible, protein-extracted, mineral-free, dilapidated, insoluble Type-I bone collagen particles which may be allergenic or xenogenic to the host. The particle size of the matrix is within the range of 70-850 μm, and the matrix may be fabricated by close packing the particles into a shape spanning the bone defect.

It is clear that Oppermann does not anticipate and/or render obvious the present invention. There is no disclosure whatsoever that the matrix material should be composed of a calcium phosphate, as claimed herein. Thus, for this reason alone, Oppermann could not anticipate or render obvious the present invention.

In addition, as with Urist, it is clear that the matrix of Oppermann is not "bioactive", as required in the present invention. It is clearly disclosed in Oppermann that the matrix alone does not induce bone growth. See, for example, page 52, lines 13-14 and page 56, lines 14-16. For this additional reason, Oppermann could not anticipate or render obvious the present invention.

Claims 1-13 are rejected under 35 USC §103(a) as being unpatentable over Urist in view of Oppermann and Hötten et al. Urist and Oppermann as been discussed above. Hötten is relied upon as disclosing the protein GDF5 (also known as MP52). The Examiner takes the position that it would have been obvious to combine Hötten with Urist and Oppermann.

As noted above, neither Urist nor Oppermann disclose that the matrix material used therein should be "bioactive" as claimed herein. The addition of Hötten would not overcome the failure of the primary references to disclose the invention. While it may have been obvious to combine MP52 with a matrix only serving as a carrier, but which exhibits the disadvantages described as the prior art, it could not have been obvious to combine MP52 with a bioactive matrix material, as claimed herein, which surprisingly does not shown the negative side effects of the known matrices. Applicants respectfully submits that the present invention is not unpatentable over Urist in view of Oppermann and Hötten.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,
Arent Fox Kintner Plotkin & Kahn

Richard J. Berman
Attorney for Applicants
Registration No. 39,107

Atty. Docket No. P564-9010
1050 Connecticut Avenue, NW
Suite 600
Washington, D.C. 20036-5339
(202) 638-5000
Enclosure: Articles (4 pages)
RJB:ccd

Bone-inducing activity of mature BMP-2b produced from a hybrid BMP-2a/2b precursor.

Hammonds RG Jr, Schwall R, Dudley A, Berkemeier L, Lai C, Lee J, Cunningham N, Raddi AH, Wood WI, Mason AJ

Department of Developmental Biology, Genentech, Inc., South San Francisco, California 94080.

The human osteoinductive proteins BMP-2a and BMP-2b have been cloned and expressed in mammalian cells. In order to improve expression levels we examined the role of the proregion in assembly and export. Use of the BMP-2a proregion combined with the mature region of BMP-2b leads to dramatically improved expression of mature BMP-2b. Mature BMP-2b has been purified to near homogeneity from the BMP-2a/2b hybrid, and its structural properties and biological activity determined. Recombinant mature BMP-2b homodimer elicits bone formation in vivo.

United States Patent; Israel et al. 5,866,364 Feb 02, 1999

Recombinant bone morphogenetic protein heterodimers

Abstract

The present invention relates to a methods for producing recombinant heterodimeric BMP proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to the recombinant heterodimers and compositions containing them.

Inventors: Israel; David (Concord, MA); Wolfman; Neil M. (Dover, MA)
Assignee: Genetics Institute, Inc. (Cambridge, MA)
Appl. No.: 989847
Filed: November 27, 1992

Auszug aus Matrixbeschreibung:

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the heterodimeric BMP compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

United States Patent
Lucas , et al.
4,931,548
June 5, 1990

Heterodimer form of transforming growth factor-beta

Abstract

A polypeptide transforming growth factor found in porcine platelets, having activity in the TGF-.beta. assay and a molecular weight of about 25 kDa. The factor is a heterodimer, one chain of which has an N-terminal sequence very different from human platelet TGF-.beta., and the other chain of which has an N-terminal sequence identical to that of human platelet TGF-.beta.. The factor is purified using gel filtration and reverse phase HPLC.

Inventors: Lucas; Roger C. (Blaine, MN); Weatherbee; James A. (St. Anthony, MN); Tsang; Monica L.-S. (St. Anthony, MN)
Assignee: Techne Corporation (Minneapolis, MN)
Appl. No.: 008808
Filed: January 30, 1987

Growth Factors 1996;13(3-4):291-300

Heterodimeric bone morphogenetic proteins show enhanced activity in vitro and in vivo.

Israel DI, Nove J, Kerns KM, Kaufman RJ, Rosen V, Cox KA, Wozney JM

Genetics Institute, Cambridge, MA 02140, USA.

The bone morphogenetic proteins (BMPs), a subgroup of the TGF-beta gene super-family, are dimeric molecules involved in the growth, differentiation and repair of a wide variety of tissues. Based on the observation that several of the BMPs co-purify when isolated from bovine bone and that a pattern of co-localization exists during mouse embryogenesis, we co-expressed various combinations of BMPs in Chinese hamster ovary cells to test for possible heterodimer formation and activity. Transient co-expression of BMP-2 with either BMP-5, BMP-6 or BMP-7, or BMP-4 transiently co-expressed with BMP-7, resulted in more BMP activity than expression of any single BMP. Stable cell lines were then made in order to purify and characterize co-expressed BMPs in more detail. Co-expression of BMP-2 with BMP-7 yielded heterodimeric BMP-2/7 with a specific activity about 20-fold higher than BMP homodimers in an in vitro alkaline phosphatase induction assay. These heterodimers were also 5- to 10-fold more potent than BMP-2 in inducing cartilage and bone in an in vivo assay. Similar results were obtained with BMP-2/6 heterodimer. These experiments demonstrate the increased potency of several BMP heterodimers relative to BMP homodimers and support the hypothesis that such heterodimeric forms are likely to have natural biological functions.

Biochem Biophys Res Commun 1995 May 25;210(3):670-7

Potent ectopic bone-inducing activity of bone morphogenetic protein-4/7 heterodimer.

Aono A, Hazama M, Notoya K, Taketomi S, Yamasaki H, Tsukuda R, Sasaki S, Fujisawa Y

Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan.

We have purified and characterized recombinant Xenopus bone morphogenetic proteins (xBMPs): homodimers of xBMP-4, 7 and heterodimers (xBMP-4/7) produced by a baculovirus expression system. Highly purified xBMPs had homogeneous NH2-termini predicted from a consensus motif, Arg-X-X-Arg, while they possessed diverse sugar chains. Implantation of xBMPs together with pure collagen carrier in rats induced new bone formation in a dose-dependent manner. The xBMP-4/7 heterodimer showed the strongest activity, with an effective dose of 1-30 micrograms, while more than 10 micrograms of xBMP-4 or 7 homodimer was required for a significant effect. Histological examination revealed that xBMP-4/7 implants showed intramembranous ossification without chondrogenesis. In primary cultures of rat bone marrow stromal cells, xBMP-4/7 induced alkaline phosphatase 3-fold more strongly than xBMP-7 and 20-fold more than xBMP-4. These results suggest that the heterodimeric form of BMP would generate the strongest signal triggering osteogenic differentiation of osteoprogenitor cells in adult tissues.

Biochem Biophys Res Commun 1995 Apr 26;209(3):859-66

Efficient expression of a heterodimer of bone morphogenetic protein subunits using a baculovirus expression system.

Hazama M, Aono A, Ueno N, Fujisawa Y

Pharmaceutical Research Division, Takeda Chemical Industries Ltd., Osaka, Japan.

Recombinant baculoviruses as expression vectors for Xenopus bone morphogenetic protein (xBMP)-2, 4 and 7 were generated. The conditioned medium of insect cells infected with the virus for xBMP-2 or 4 showed strong alkaline phosphatase-inducing activity in a mouse osteoblastic cell line, MC3T3-E1, although a large portion of the activity remained in the infected cells. In contrast, xBMP-7 was preferentially secreted into the medium, but had only weak activity. Conditioned media following simultaneous inoculation with the viruses for xBMP-7 and for xBMP-2 or 4 showed a remarkably increased level of activity. The increased activity was clearly separated from other peaks derived from single infection on a cation-exchange column and was found to arise from the disulfide-linked heterodimer consisting of xBMP-4 and 7 subunits by immunoblot analysis. The heterodimer also augmented osteocalcin production and parathyroid hormone-sensitivity more strongly than the homodimers. These results suggest that our expression system provides a convenient source of heterodimeric BMP with high osteogenic differentiation-inducing activity.

J Biol Chem 1990 Aug 5;265(22):13198-205

Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily.

Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, Ozkaynak E, Oppermann H, Rueger DC

Creative BioMolecules, Inc., Hopkinton, Massachusetts 01748.

A bone-inductive protein has been purified from bovine bone and designated as osteogenic protein (OP). The purified OP induces new bone at less than 5 ng with half-maximal bone differentiation activity at about 20 ng/25 mg of matrix implant in a subcutaneous bone induction assay. The purified osteogenic protein is composed of disulfide-linked dimers that migrate on sodium dodecyl sulfate gels as a diffuse band with an apparent molecular weight of 30,000. Upon reduction, the dimers yield two subunits that migrate with molecular weights of 18,000 and 16,000. Both subunits are glycosylated. After chemical or enzymatic deglycosylation, the dimers migrate as a diffuse 27-kDa band that upon reduction yields two polypeptides that migrate at 16 kDa and 14 kDa, respectively. The carbohydrate moiety does not appear to be essential for biological activity since the deglycosylated proteins are capable of inducing bone formation in vivo. Amino acid sequences of peptides generated by proteolytic digestion show that the subunits are distinct but related members of the transforming growth factor-beta super-family. The 18-kDa subunit is the protein product of the bovine equivalent of the human OP-1 gene and the 16-kDa subunit is the protein product of the bovine equivalent of the human BMP-2A gene.

J Biol Chem 1992 Feb 5;267(4):2325-8

Purification and characterization of transforming growth factor-beta 2.3 and -beta 1.2 heterodimers from bovine bone.

Ogawa Y, Schmidt DK, Dasch JR, Chang RJ, Glaser CB

Celtrix Laboratories, Palo Alto, California 94303.

A unique form of transforming growth factor-beta (TGF-beta), TGF-beta 2.3 heterodimer, has been purified from bovine bone extract. TGF-beta 2.3 migrated as a single 25-kDa band by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, whereas under reducing conditions it migrated as a 12.5 kDa band. The TGF-beta 2.3 reacted positively with anti-TGF-beta 2 and anti-TGF-beta 3 antibodies on immunoblots. Equal levels of TGF-beta 2 and TGF-beta 3 sequences were detected by N-terminal sequencing. TGF-beta 2.3 eluted as a single sharp peak by reverse-phase high performance liquid chromatography. However, prior reduction of the protein with dithiothreitol resulted in the protein eluting in two peaks, one containing predominantly TGF-beta 3 and the other containing predominantly TGF-beta 2. TGF-beta 2.3 inhibited proliferation of mink lung epithelial cells and promoted the formation of colonies of normal rat kidney fibroblasts in culture with specific biological activity similar to those of TGF-beta 1 and TGF-beta 2. These results demonstrate that the protein is TGF-beta 2.3 heterodimer, consisting of one polypeptide chain each of TGF-beta 2 and TGF-beta 3 linked by one or more disulfide bonds. In addition, TGF-beta 1.2 heterodimer, previously found only in porcine platelets, has also been purified from bovine bone extract.

Nature 1986 Jun 19-25;321(6072):779-82

Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin.

Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R

Inhibin is a gonadal protein that specifically inhibits the secretion of pituitary follicle-stimulating hormone (FSH). Two forms of inhibin (A and B) have been purified from porcine follicular fluid and characterized as heterodimers of relative molecular mass (Mr) 32,000 (ref. 2). Each inhibin is comprised of an identical alpha-subunit of Mr 18,000 and a distinct but related beta-subunit of Mr 13,800-14,700 linked by interchain disulphide bond(s). Throughout the purification of inhibins, we consistently observed two fractions which stimulated the secretion of pituitary FSH. We report here the isolation of one of the FSH-releasing proteins; it has a Mr of 24,000 and its N-terminal sequences up to residue 32 are identical to those of each beta-subunit of inhibins A and B. In the presence of reducing agents, SDS-polyacrylamide gel electrophoresis resolves the FSH-releasing substance into two subunits which are identical in their migration behaviour to the reduced beta-subunits of inhibins A and B. Based on the N-terminal sequence data and Mr of the intact and reduced molecules, we propose that the FSH-releasing substance, which is active in picomolar concentrations, is a heterodimeric protein composed of the two beta-subunits of inhibins A and B linked by interchain disulphide bond(s). The structural organization of the FSH-releasing substance is homologous to that of transforming growth factor-beta (TGF-beta), which also posseases FSH-releasing activity in the same bioassay. We suggest that the substance be called activin to signify the fact that it has opposite biological effects to inhibin.